173. *Studies in Relation to Biosynthesis. Part XXII.*[1] *Palitantin and Cyclopaldic Acid.*

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Palitantin (IV), a metabolite of *Penicillium cyclopium*, was produced by growth on a medium containing $Me\cdot{}^{14}CO_2Na$. Degradation of the radioactive substance shows that it is built up by the head-to-tail linkage of acetic acid units. Cyclopaldic acid (X), produced by the same organism, has a labelling-pattern in accord with the origin of its nucleus from four acetic acid and two C_1 units.

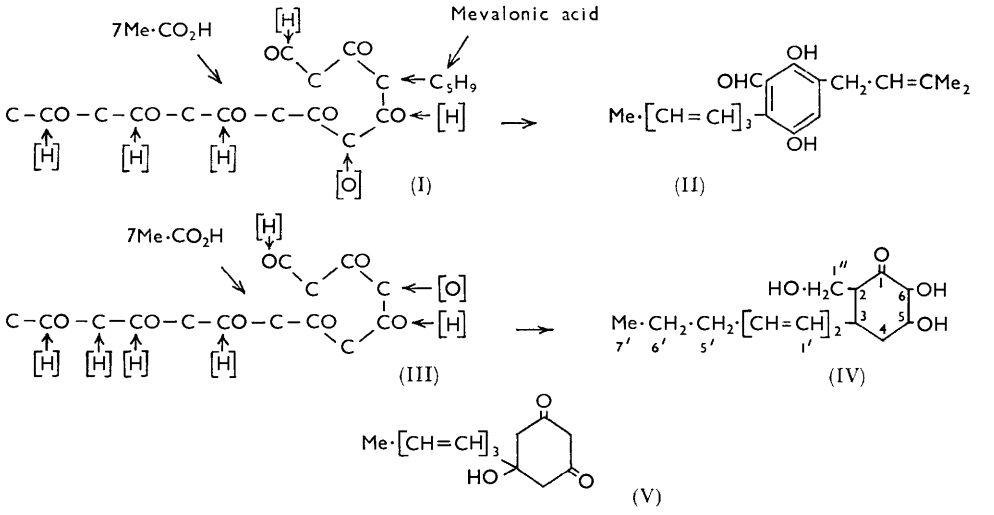
Several mould metabolites containing both aliphatic and aromatic groups in their molecules have been shown to arise by the head-to-tail linkage of acetic acid units [1,2] but at present little evidence is available about intermediates in such syntheses. Biosynthesis of such compounds can be conveniently considered under two headings for purposes of investigation: (i) production of the main skeleton from acetic acid units, and (ii) further structural changes, including the introduction of C_1 and C_5 groups,[3,4] aromatisation, and introduction or removal of oxygen. Investigation of stage (i) appears at present to be feasible only by strictly biochemical techniques, but much information on stage (ii) should be obtainable by studies of intermediates of about the same molecular size as the final products.

Among other approaches to these problems we have sought cyclic compounds which might be related to precursors of aromatic compounds, and substances which might be suitable for study of the oxidative metabolism of intermediates. We report now some

[1] Part XXI, *Tetrahedron*, 1959, **7**, 241.
[2] Birch and Donovan, *Austral. J. Chem.*, 1953, **6**, 360; Birch, Massy-Westropp, and Moye, *ibid.*, 1955, **8**, 539; Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.
[3] Birch, English, Massy-Westropp, Slaytor, and Smith, *J.*, 1958, 365.
[4] Birch, Schofield, and Smith, *Chem. and Ind.*, 1958, 1321.

work on palitantin which is in the former category, and cyclopaldic acid which is in the latter.

Palitantin.—Recent studies [4] on the biosynthesis of auroglaucin (II) indicate operation of the scheme (I) ⟶ (II), the core of the molecule being a C_{14} compound into which has been introduced an isopentane unit. A non-aromatic cyclic C_{14} compound known to contain a C_7 side-chain is palitantin,[5] the structure of which was incompletely known when



this work was projected. Before beginning experimental work we learned that Professor B. Lythgoe, F.R.S., was engaged on the structural problem and our work was postponed until his was complete, showing the structure to be (IV).[6] We are very grateful to Professor Lythgoe for information in advance of publication. The degradations employed were either from the original work [5] or from that of Lythgoe,[6] with some additions noted in the Experimental section.

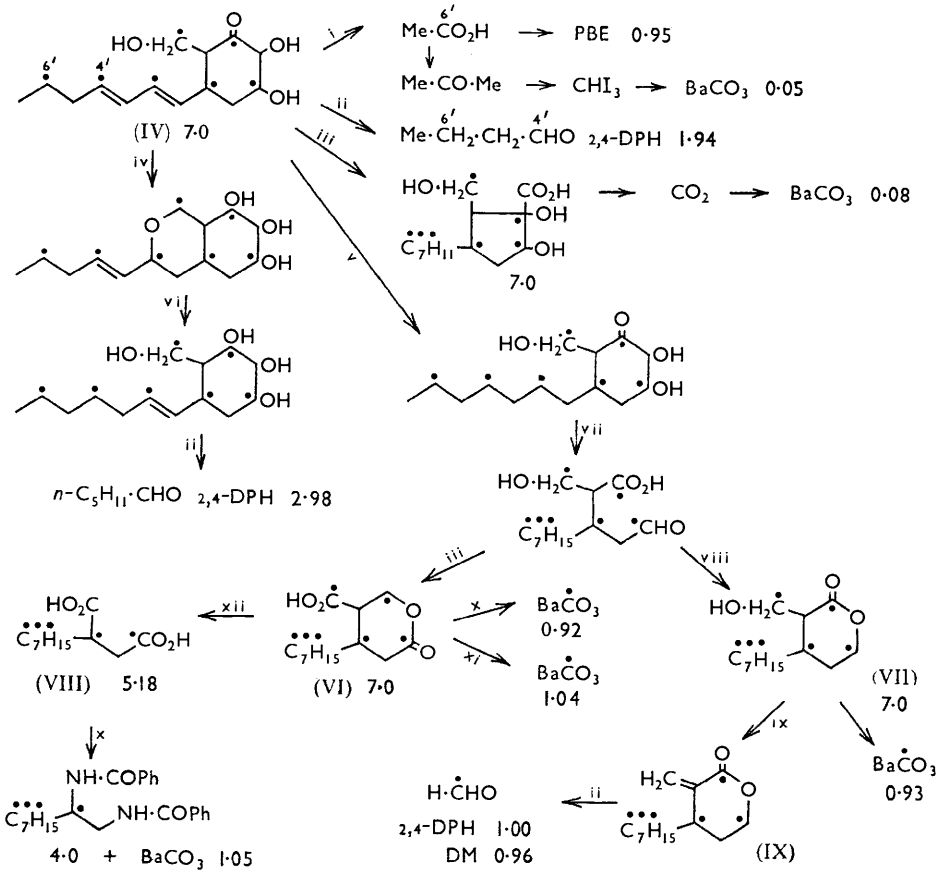
The structural similarities between structures (II) and (IV) can be rationalised on the biogenetic conversions (I) ⟶ (II) and (III) ⟶ (IV), which would indicate that the two substances are branches of a common scheme which probably includes a C_{14} compound such as (V). Palitantin itself is unlikely to be an intermediate of auroglaucin biosynthesis. A notable similarity is that in both cases the reduction of one of the carbonyl groups in the hypothetical precursor (V) must be postulated to account for the presence of a hydroxyl in its place in palitantin (IV) and for the loss of an expected hydroxyl group *meta* to the side chain in auroglaucin (II). Auroglaucin is accompanied by flavoglaucin with a saturated C_7 side chain, so the reduction of this chain, as in (III) ⟶ (IV), obviously could occur readily. With these ideas in mind further approaches to the problem are possible and are being pursued.

The first step necessary to support such ideas is to show that palitantin, like auroglaucin, arises from acetic acid units, a possibility also noted by Lythgoe and his colleagues.[6] The strain of *Penicillium cyclopium*, obtained from Professor Lythgoe, was grown on a medium containing $Me\cdot{}^{14}CO_2Na$ and the palitantin was isolated and degraded as shown in scheme A, the results being expressed in our usual manner.[4] In this case the relative molecular activity (r.m.a.) $\times 10^{-5}$ values permit the number of labelled carbon atoms per molecule to be read directly. The assumption of alternate labelled atoms is

[5] Birkinshaw and Raistrick, *Biochem. J.*, 1936, **30**, 801; Birkinshaw, *ibid.*, 1952, **51**, 271.
[6] Bowden, Lythgoe, and Marsden, *J.*, 1959, 1662.

supported, not only by the fit of all the results, but also by the fact that it is possible either to determine directly or to calculate that the 1-, 3-, and 7′-positions contain no ^{14}C. Assuming that this distribution holds completely makes it possible to calculate the activity of each labelled atom (r.m.a. $\times 10^{-5}$, theoretical = 1).

SCHEME A



Reagents: (i) CrO_3. (ii) O_3. (iii) HgI_2. (iv) (*a*) KBH_4, (*b*) I_2. (v) Pd–H_2. (vi) (*a*) Pd–H_2, (*b*) Zn–AcOH. (vii) KIO_4. (viii) KBH_4. (ix) (*a*) Me·C_6H_4·SO_2Cl, (*b*) pyridine. (x) HN_3. (xi) Heat. (xii) HNO_3.

Abbreviations: 2,4-DPH 2,4-Dinitrophenylhydrazone. DM Dimedon derivative. PBE *p*-Bromophenacyl ester.

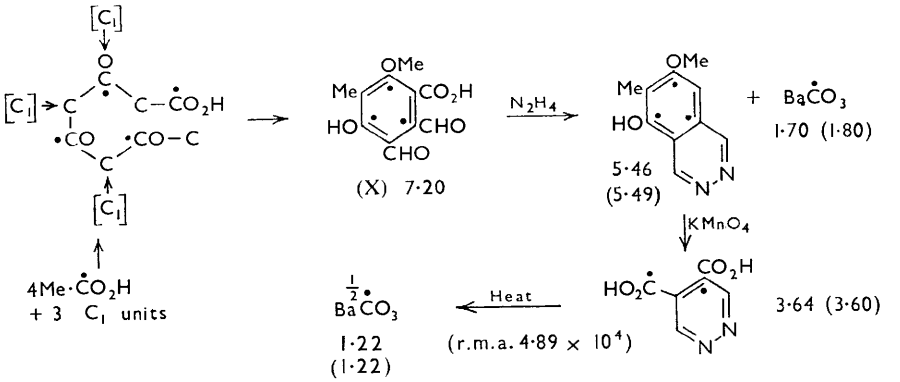
Numerals denote relative molecular activities $\times 10^{-5}$.

Cyclopaldic Acid.—This substance (X), which occurs in larger amounts in other strains of *P. cyclopium* (Westling),[7] was a minor product with our strain, but sufficient was obtained from media containing Me·$^{14}CO_2H$ to support the expected distribution of labelling, as shown in (X). The degradations carried out are shown in scheme B (theoretical figures in parentheses). Although it is not yet finally confirmed, the nuclear methyl and one aldehyde group are therefore probably introduced C_1 units.[3] The substance therefore raises a number of problems connected with the stages at which methylations and oxidations occur, and with the nature of the acetic acid-derived side

[7] Raistrick, Raistrick, Ross, and Stickings, *Biochem. J.*, 1951, **50**, 610.

C_6' as *p*-Br·C_6H_4·CO·CH_2·O_2C·CH_3	0·95
$C_6' + C_4'$ as butyraldehyde 2,4-dinitrophenylhydrazone	1·94
Average Ċ	0·97
C_4' by difference	0·99
$C_6' + C_4' + C_2'$ as n-hexanal 2,4-dinitrophenylhydrazone	2·98
Average Ċ	0·99
C_2' by difference	1·04
$C_6' + C_4' + C_2' + C_3$ as *NN'*-dibenzoylheptylethylenediamine	4·00
Average Ċ	1·00
C_3 by difference	1·02
$C_6' + C_4' + C_2' + C_3 + C_5$ as n-heptylsuccinic acid	5·18
Average Ċ	1·05
C_5 by difference	1·18
C_5 by HN_3 on (VIII)	1·04
C_1 from (VI)	0·92, 1·04
C_1 from (VII)	0·93
C_1'' from (IX)	1·00, 0·96
$C_1 + C_1''$ (palitantin — n-heptylsuccinic acid)	1·82

SCHEME B



chain which appears as CHO adjacent to CO_2H. These problems may be attacked by feeding experiments involving potential intermediates, and such work is in progress.

EXPERIMENTAL

[^{14}C]*Palitantin.*—*Penicillium cyclopium* was grown as described in the literature.[6] After 17 days at 24° the extracted material [6] (200 mg./l.) had m. p. 163—165°. In a similar culture Me·$^{14}CO_2Na$ [0·2 mc in water (10 c.c.), distributed between 10 penicillin flasks in a total of 1·5 l.] gave rise to [^{14}C]palitantin (1·8%, 9·64 μc). For the degradations, material of r.m.a. 300—450 × 10^5 was used. For simplicity, all the r.m.a. values below have been referred to a palitantin of r.m.a. 7 × 10^5.

Degradations of Palitantin.—Kuhn–Roth oxidation gave acetic acid, whose *p*-bromophenacyl ester, m. p. 84—86°, had r.m.a. × 10^{-5}, 0·95. A portion of the acetic acid was pyrolysed as lithium acetate, and the resulting acetone was converted into iodoform and thence into barium carbonate of negligible activity (Found: r.m.a. × 10^{-5}, 0·05).

Ozonolysis of palitantin (0·25 g.) in chloroform (10 c.c.) at 0° gave butyraldehyde, converted into its 2,4-dinitrophenylhydrazone, m. p. 123—124° (Found: r.m.a. × 10^{-5}, 1·94).

Palitantic acid [5] (Found: r.m.a. × 10^{-5}, 7·0) (25 mg.) was refluxed in quinoline (5 c.c.) with copper bronze (50 mg.) under nitrogen for 2 hr., the resulting carbon dioxide being absorbed in saturated barium hydroxide solution. The activity of the resulting barium carbonate was negligible (Found: r.m.a. × 10^{-5}, 0·08).

Tetrahydropalitantin was oxidised to the $C_{13}H_{22}O_4$ lactone acid (VI) of Raistrick and Birkinshaw [5] which in our hands crystallised from ether–pentane as colourless prisms, m. p. 68—69° (Found: C, 64·55; H, 9·05%; r.m.a. × 10^{-5}, 7. Calc. for $C_{13}H_{22}O_4$: C, 64·5; H, 9·1%; r.m.a. × 10^{-5}, 7). The infrared spectrum included maxima at 3225, 2905, 2837, 1710, 1379, and 1185 cm.$^{-1}$. Pyrolysis gave rather a poor yield of barium carbonate (r.m.a. × 10^{-5}, 1·04), and Schmidt degradation gave also rather a small yield of barium carbonate (r.m.a. × 10^{-5}, 0·92).

The $C_{13}H_{22}O_4$ lactone-acid (VI) (500 mg.) was oxidised with concentrated nitric acid as described,[5] giving n-heptylsuccinic acid (150 mg.), m. p. 74—76° (Found: r.m.a. × 10^{-5}, 5·18). The succinic acid (60 mg.) and phosphorus oxychloride (30 mg.) were heated at 200° for 10 min. After cooling, fuming sulphuric acid (*d* 1·86; 0·25 c.c.) was added and dissolution completed by shaking and gentle warming. To this mixture, at 0°, powdered sodium azide (50 mg.) was quickly added and the flask immediately attached to an acid potassium permanganate trap and a trap containing saturated barium hydroxide solution. Nitrogen was then passed through the mixture, which was allowed to warm to room temperature and then heated to 60—80°. After 15 min. precipitation was complete of the barium hydroxide (Found: 2 × r.m.a. × 10^{-5}, 1·05). The acidic residue was diluted with water (10 c.c.), made alkaline with concentrated aqueous potassium hydroxide, and extracted with ether (altogether 50 c.c.). The ether solution was dried (K_2CO_3) and the ether removed. The residue was converted into the *NN'*-dibenzoyl derivative by reaction with benzoyl chloride (0·1 c.c.) in pyridine (0·5 c.c.). Worked up in the usual way the *NN'*-dibenzoyl derivative of n-heptylethylenediamine was obtained. It was difficult to crystallise and was sublimed in a vacuum, then having m. p. 166—168° [Found: C, 75·3; H, 8·05%; r.m.a. × 10^{-5}, 4·0. $C_{23}H_{30}O_2N_2$ requires C, 75·4; H, 8·25%; r.m.a. × 10^{-5} (4C), 4·0].

Tetrahydropalitantin was converted [6] into the $C_{13}H_{24}O_3$ lactone (VII), m. p. 46° (Found: r.m.a. × 10^{-5}, 7·0). Yields of carbon dioxide were low on pyrolysis of the lactone in quinoline, but the activity of the barium carbonate was the same (Found: r.m.a. × 10^{-5}, 0·93) as that obtained on pyrolysis of the sodium salt of the free acid. The $C_{13}H_{24}O_3$ lactone was converted *via* the toluene-*p*-sulphonate into the unsaturated lactone,[6] $C_{13}H_{22}O_2$, which was ozonised in ethyl acetate–acetic acid solution. The resulting formaldehyde was measured as the 2,4-dinitrophenylhydrazone (Found: r.m.a. × 10^{-5}, 1·0) and the dimedone derivative (Found: r.m.a. × 10^{-5}, 0·96).

Palitantol [6] (0·62 g.) was converted by iodination, hydrogenation, and de-iodination [6] into dihydropalitantol (Found: r.m.a. × 10^{-5}, 7·0). This was ozonised at 0° in ethyl acetate–acetic acid solution to give n-hexanal 2,4-dinitrophenylhydrazone, m. p. 100—103° (Found: r.m.a. × 10^{-5}, 2·98).

Cyclopaldic Acid.—The mother-liquors after crystallisation of the palitantin were evaporated and redissolved in ether. After several days the crystalline precipitate was removed, dissolved in ethyl acetate, and taken up in potassium hydrogen carbonate solution. The aqueous solution was treated with charcoal, acidified, and extracted with ethyl acetate, and the cyclopaldic acid crystallised from aqueous ethanol. It was finally purified by vacuum-sublimation and crystallised from ethanol, then having m. p. 224—226° (Found: C, 55·75; H, 4·3. Calc. for $C_{11}H_{10}O_6$: C, 55·45; H, 4·2%). It gave the reported colour reactions and had $\lambda_{max.}$ 245, 278, 322 mμ (log ε 4·57, 4·04, 3·41). It gave a tetra-acetyl ester, m. p. 159—161° (lit., 159°). Isotopically labelled acid was obtained from the metabolic solutions containing Me·$^{14}CO_2H$ (incorporation 0·2%).

Degradation to 5-hydroxy-7-methoxy-6-methylphthalazine. Cyclopaldic acid (70 mg.) (r.m.a. × 10^{-5}, 7·2) was converted as previously described into the phthalazine (45 mg.), m. p. 259—263° [Found: r.m.a. × 10^{-5}, 5·46 (3C, 5·49)] and carbon dioxide, converted into barium carbonate (56 mg.) [Found: r.m.a. × 10^{-5}, 1·70 (1C, 1·8)].

Oxidation of the phthalazine to pyridazine-4,5-dicarboxylic acid. The general process followed the oxidation of phthalazine by Gabriel.[8] The above phthalazine (45 mg.) in N-aqueous sodium hydroxide (0·8 c.c.) and water (3 c.c.) was heated on the steam-bath and 2·5% potassium permanganate solution was added drop by drop until its colour persisted (6·5 c.c.). A small amount of ethanol was added, the solution filtered, and the filtrate evaporated under reduced pressure. The solid was treated with N-hydrochloric acid (3 c.c.). The phthalazinedicarboxylic acid separated. When recrystallised, it had m. p. 208—210° (23 mg.) [Found: r.m.a. × 10^{-5},

[8] Gabriel, *Ber.*, 1903, **36**, 3373.

3·64 (2C, 3·6)]. At this stage it was diluted with inactive material to r.m.a. $4·89 \times 10^{-4}$. This acid (30 mg.) was decarboxylated by heating it with copper bronze (100 mg.) in quinoline (5 c.c.) under nitrogen. The carbon dioxide was converted into barium carbonate (81 mg.) [Found: r.m.a. $\times 10^{-4}$, 1·22 (0·5C, 1·22)].

We are grateful to Dr. Herchel Smith for assistance with the tracer measurements and for helpful discussions, to the Polish Ministry of Higher Education for a Fellowship (to M. K.), to Imperial Chemical Industries Limited for the loan of tracer equipment, and to the Rockefeller Foundation and to the Distillers' Company Limited for financial assistance.

THE UNIVERSITY, MANCHESTER, 13. [*Received, May 22nd,* 1959.]